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Analytix

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You Deserve Brighter Analysis

Dr. Gerd Battermann

Dear Reader,

Instrumental analytical techniques like volumetric titration, Karl Fischer titration, HPLC, LC-MS, GC, atomic spectroscopy and also classical analytical techniques are only as reliable as the quality of the solvents and inorganics used. We have brought together the exceptional knowledge, products and capabilities of Merck Millipore and Sigma-Aldrich® so you can continue to trust that your results will have the highest sensitivity and reliability. A global network of R&D, manufacturing, and quality assurance combined with centuries of experience gives you the most up-to-date method development and documentation support for solvents and inorganics. Our close cooperation with you, our customers, allows us to develop and support innovative solutions, not just products. Easy ordering on **sigma-aldrich.com** and our worldwide distribution organization mean you can find the right solution and have it delivered the way you want it.

Our complete portfolio of Solvents, Inorganics & Safety Essentials is available on **sigma-aldrich.com**. Now that's convenient.

Kind regards,

Dr. Gerd Battermann
Head of Instrumental Analysis Franchise
Life Science | Applied Solutions SMI | Advanced Analytical

Titripac® – Good for the Environment, Good for the Lab

Titripac – Winner of the Green Good Design Award 2016

Bettina Straub-Jubb, Global Product Manager Titration
bettina.straub-jubb@merckgroup.com

The innovative and sophisticated Titripac was awarded the Green Good Design Award 2016 for its sustainability and eco-friendly design.

It is a joint award from The European Centre for Architecture Art Design and Urban Studies and The Chicago Athenaeum: Museum of Architecture and Design.

GOOD DESIGN™ was created in 1950 by Ereo Saarinen, Charles and Ray Eames and Edgar Kauffmann, Jr. to foster and promote modern design for architecture, industry and products. In 2009, the Green Good Design program established a special award for innovative design with sustainable properties for the environment.

Titripac significantly contributes to the reduction of packaging waste and laboratory chemical waste, saves storage space, and cuts emissions from shipping and waste.

Economic and Ecological Advantages of Using Titripac in Your Lab

You can optimize your work process in the laboratory when using volumetric solutions, buffer solutions or any other aqueous solutions in Titripac.

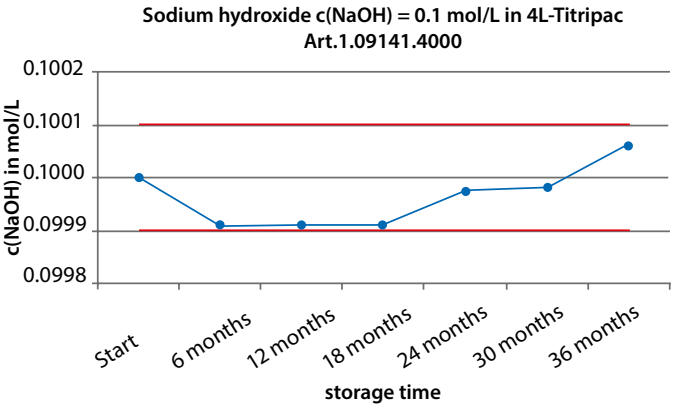
Titripac is a hermetically sealed packaging system which ensures the consistent quality of a volumetric solution or buffer solution from the first to the last drop, even while the package is in use. Contamination caused by ambient air, carbon dioxide and microbes is prevented. It eliminates time-consuming processes required to check the solutions and also reduces laboratory chemical waste, and chemical and packaging waste. It saves storage space and cuts emissions from shipping and waste.

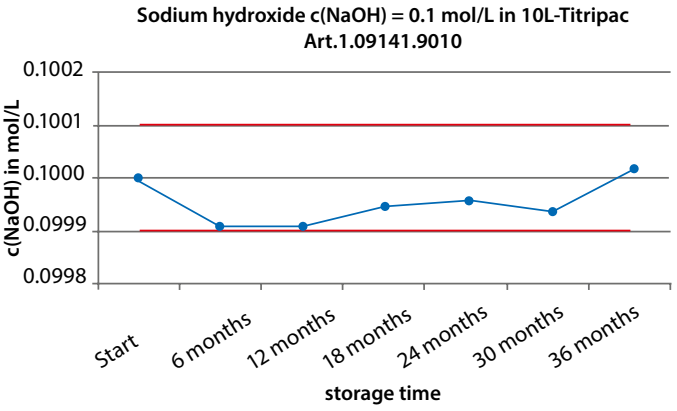
Titripac is extremely easy to use. The integrated spout appears simply by pressing on the pack. By opening the tap, liquid can be withdrawn at any time – conveniently and without the risk of contamination. In addition, Titripac can be connected directly to titrators with an adapter and a hose.

Stability Data of Titripac Sodium Hydroxide Solution

Stability was tested under daily routine conditions over a 36-month period. Every six months, the concentration of the sodium hydroxide solution was determined by titration with hydrochloric acid standard solution (item number 1.09060), which was standardized against volumetric standard tris(hydroxymethyl)aminomethane (item number 1.02408).

The following diagrams show the measured concentration of sodium hydroxide solution (item number 1.09141) in a Titripac 4 L and 10 L over a shelf life of 36 months.





Reduce Waste Using the Innovative Titripac Instead of PE Bottles

Reduce Impact to the Environment Using Titripac

Our Titripac is designed to offer the highest level of innovation, quality, safety, and effectiveness, while at the same time helping to minimize environmental impacts associated with their use. We aim to develop future-oriented products and solutions that meet performance needs, resulting in reduced life cycle impacts and helping to solve global sustainability challenges.

Product improvement highlights

MATERIALS
42%
reduction in packaging material mass[1]

EMISSIONS & ENERGY
42%
reduction in life cycle greenhouse gas emissions[1]

PACKAGING & DISTRIBUTION
35%
reduction in greenhouse gas emissions for distribution to US customer[1]

WASTE
73%
reduction in solid waste mass at user facility[1]

1 Compared to our product delivery system using 1 liter bottles

For more information on our product sustainability and Corporate Responsibility initiatives, please visit:
www.merckmillipore.com/responsibility

Titripac Features and Advantages

- Reduces environmental impact of disposal – less packaging waste, as outer cardboard box and inner bag can be disposed of separately
- Saves costs and time – no unnecessary re-testing of the solution
- Reliable to use to the last drop – hermetically sealed pack, no contaminated residual amounts and less waste
- Easy to use – integrated withdrawal tap, direct connection to instruments

Discover more on **sigma-aldrich.com/titripac**

Cat. No.	Description	Concentration	Package Size
1.09060.4000	Volumetric solutions for titration	0.1 mol/L (0.1 N)	4 L
1.09060.9010	Titripur® hydrochloric acid solution	0.1 mol/L (0.1 N)	10 L
1.13136.9010	Titripur hydrochloric acid solution	0.357 mol/L (1/2.8 N)	10 L
1.09058.4000	Titripur hydrochloric acid solution	0.5 mol/L (0.5 N)	4 L

Cat. No.	Description	Concentration	Package Size
1.09057.4000	Titripur hydrochloric acid solution	1 mol/L (1 N)	4 L
1.09057.9010	Titripur hydrochloric acid solution	1 mol/L (1 N)	10 L
1.13134.9010	Titripur hydrochloric acid solution	3.571 mol/L (1/0.28 N)	10 L
1.09081.4000	Titripur hydrochloric acid solution	0.1 mol/L (0.1 N)	4 L
1.09081.9010	Titripur silver nitrate solution	0.1 mol/L (0.1 N)	10 L
1.09041.4000	Titripur silver nitrate solution	0.1 mol/L (0.1 N)	4 L
1.09041.9010	Titripur sodium hydroxide solution	0.1 mol/L (0.1 N)	10 L
1.09140.9010	Titripur sodium hydroxide solution	0.2 mol/L (0.2 N)	10 L
1.09139.9010	Titripur sodium hydroxide solution	0.25 mol/L (0.25 N)	10 L
1.05595.9010	Titripur sodium hydroxide solution	0.33 mol/L (0.33 N)	10 L
1.09138.4000	Titripur sodium hydroxide solution	0.5 mol/L (0.5 N)	4 L
1.09138.9010	Titripur sodium hydroxide solution	0.5 mol/L (0.5 N)	10 L
1.09137.4000	Titripur sodium hydroxide solution	1 mol/L (1 N)	4 L
1.09137.9010	Titripur sodium hydroxide solution	1 mol/L (1 N)	10 L
1.09147.4000	Titripur sodium hydroxide solution	0.1 mol/L (0.1 N)	4 L
1.09147.9010	Titripur sodium thiosulfate solution	0.1 mol/L (0.1 N)	10 L
1.09074.4000	Titripur sodium thiosulfate solution	0.05 mol/L (0.1 N)	4 L
1.09074.9010	Titripur sulfuric acid solution	0.05 mol/L (0.1 N)	10 L
1.09073.4000	Titripur sulfuric acid solution	0.25 mol/L (0.5 N)	4 L
1.09073.9010	Titripur sulfuric acid solution	0.25 mol/L (0.5 N)	10 L
1.09072.4000	Titripur sulfuric acid solution	0.5 mol/L (1 N)	4 L
1.09072.9010	Titripur sulfuric acid solution	0.5 mol/L (1 N)	10 L
1.08420.9010	Titripur sulfuric acid solution	10 mg CaO/L = 1 mL	10 L
1.08431.4000	Titriplex® solution B for the determination of water hardness	0.1 mol/L (0.1 N)	4 L
1.08431.9010	Titriplex III solution (Na_2-EDTA)	0.1 mol/L (0.1 N)	10 L
1.08447.4000	Titriplex III solution (Na_2-EDTA)	0.1 mol/L (0.1 N)	4 L
Buffer solutions for pH measurement			
1.09433.4000	Certipur® buffer solution	pH 2.00 (20 °C)	4 L
1.09433.9010	Certipur buffer solution	pH 2.00 (20 °C)	10 L
1.09435.4000	Certipur buffer solution	pH 4.00 (20 °C)	4 L
1.09435.9010	Certipur buffer solution	pH 4.00 (20 °C)	10 L
1.09437.4000	Certipur buffer solution	pH 6.00 (20 °C)	4 L
1.09439.4000	Certipur buffer solution	pH 7.00 (20 °C)	4 L
1.09439.9010	Certipur buffer solution	pH 7.00 (20 °C)	10 L
1.09460.4000	Certipur buffer solution	pH 8.00 (20 °C)	4 L
1.09461.4000	Certipur buffer solution	pH 9.00 (20 °C)	4 L
1.09461.9010	Certipur buffer solution	pH 9.00 (20 °C)	10 L
1.09438.4000	Certipur buffer solution	pH 10.00 (20 °C)	4 L
1.09438.9010	Certipur buffer solution	pH 10.00 (20 °C)	10 L
1.09475.4000	Certipur buffer solution colored red	pH 4.00 (20 °C)	4 L
1.09475.9010	Certipur buffer solution colored red	pH 4.00 (20 °C)	10 L
1.09477.4000	Certipur buffer solution colored green	pH 7.00 (20 °C)	4 L
1.09477.9010	Certipur buffer solution colored green	pH 7.00 (20 °C)	10 L
1.09476.4000	Certipur buffer solution colored blue	pH 9.00 (20 °C)	4 L
1.09476.9010	Certipur buffer solution colored blue	pH 9.00 (20 °C)	10 L
Other products			
1.16754.4000	Emsure® water for analysis		4 L
1.16754.9010	Emsure water for analysis		10 L
1.00496.9010	Formaldehyde solutions 4%, buffered for histology	pH 6.9	10 L
1.01728.9010	Osteosoft® mild decalcifier solution for histology		10 L
4.80370.9010	Phosphate buffer solution	pH 2.5 (25 °C)	10 L

Table 1. Ordering Information for Products in Titripac

Brighter Water Determination with Aquastar™ Reagents for Brilliant Karl Fischer Titration Results

Aquastar – Our New Global Karl Fischer Product Line

Bettina Straub-Jubb, Global Product Manager Titration
bettina.straub-jubb@merckgroup.com

We are proud to present our new global product range of Karl Fischer reagents and standards named Aquastar. We will replace our existing Apura® trademark with the new brand name Aquastar outside of North America. In North America, Aquastar is already a very well known and established high-quality brand. To offer one globally available product line, all Karl Fischer products will use the brand name Aquastar in the future.

All products will keep the same catalog numbers, designation, composition, quality and performance. Outside of the United States and Canada, we will just change the brand name to Aquastar – nothing more. A few Karl Fischer products branded in North America with Apura will also be re-branded to Aquastar.

Aquastar – Quality You Can Count On

Karl Fischer titration is a widely used method for water determination. The significance of determining water content in raw materials, intermediates and finished products is underscored by the fact that water influences the properties of substances. Shelf life, stability, agglomeration, physical properties, etc. will all be affected by too much water.

By using Karl Fischer titration together with our Aquastar reagents and standards, the water content of gases, liquids and solids can easily be determined with a high degree of accuracy.

As we apply the highest standards to production processes and comply with stringent testing requirements, Aquastar reagents are distinguished by excellent quality. Measurement results are therefore always reliable. From the selection of raw materials and packaging on through quality control, we apply the strictest criteria.

You can always trust your water content results with Aquastar reagents and standards.

Advantages

- High accuracy and excellent precision
- Rapid and reproducible titration results
- Large water capacity
- Comprehensive product line for any application
- Excellent range of water standards

Benefit from More than 40 Years of Experience

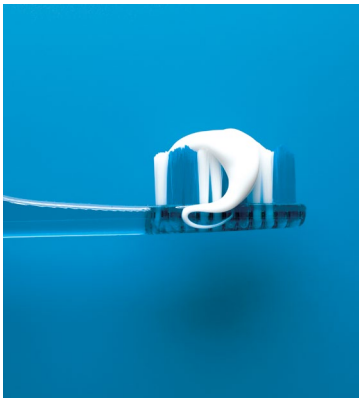
With more than 40 years of experience in continually developing and improving Karl Fischer reagents and standards, developing new applications in our application laboratories in the U.S., Europe and Asia, and supporting our customers in their daily lab work, you can count on our impressive in-house level of expertise.

We Support You

With our regular Karl Fischer seminars and in-house trainings, we educate our customers and bring them the latest updates in Karl Fischer titration.

Discover our application notes under:
sigma-aldrich.com/kf-applications

For further support or interest regarding Karl Fischer seminars, please contact us at:
Aquastar@merckgroup.com outside of North America
Aquastar@milliporesigma.com in North America



Cat. No.	Description	Packaging
One Component Reagents		
1.88005	Aquastar™ – CombiTitrant 5 approx. 5 mg H_2O/mL	Glass bottle
1.88002	Aquastar – CombiTitrant 2 approx. 2 mg H_2O/mL	Glass bottle
1.88001	Aquastar – CombiTitrant 1 approx. 1 mg H_2O/mL	Glass bottle
1.88008	Aquastar – CombiSolvent, methanol-free solvent	Glass bottle
1.88009	Aquastar – CombiMethanol	Glass bottle
Two Component Reagents		
1.88010	Aquastar – Titrant 5 approx. 5 mg H_2O/mL	Glass bottle
1.88011	Aquastar – Titrant 2 approx. 2 mg H_2O/mL	Glass bottle
1.88015	Aquastar – Solvent	Glass bottle
Solvents for Oils and Fats		
1.88020	Aquastar CombiSolvent fats (for fats in foodstuff) Use together with CombiTitrant	Glass bottle
1.88021	Aquastar CombiSolvent oils (for mineral oils) Use together with CombiTitrant	Glass bottle
1.88016	Aquastar Solvent oils & fats solvent for long-chained substances Use together with CombiTitrant or Titrant	Glass bottle

Cat. No.	Description	Packaging
Reagents for Aldehydes and Ketones		
1.88006	Aquastar CombiTitrant 5 Keto	Glass bottle
1.88007	Aquastar CombiSolvent Keto	Glass bottle
Reagents for Coulometry		
1.09255	Aquastar CombiCoulomat frit For cells with diaphragm One reagent for anode and cathode	Glass bottle
1.09257	Aquastar CombiCoulomat fritless For cells with and without diaphragm One reagent for anode and cathode	Glass bottle
Buffer Solutions		
1.88035	Aquastar Buffer solution for strong acids	Glass bottle
1.88036	Aquastar Buffer solution for strong bases	Glass bottle

Table 1. Ordering Information

More Reliable Results Thanks to Organic Certified Reference Materials

New CRMs for Chromatography and ^{1}H and ^{31}P Quantitative NMR

Matthias Nold, Product Manager Reference Materials
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There are many factors that determine the quality of an analytical measurement. Reliable analytical methods, sophisticated instruments and modern laboratory equipment are as important as the experience and proficiency of the scientists who perform the measurements. However, even if all these requirements are fulfilled, the reliability of the analytical measurement still ultimately depends on the reliability of the reference material that is used for calibration. Thus, trusting your analytical results means trusting your reference material.

Traceability of your reference material to an international standard (such as a reference material from NIST) is an effective means of achieving highly reliable and comparable results. However, for organic molecules, it is often not easy to find a traceable reference material. Most commercially available reference materials are not traceable to an internationally accepted standard since, for most analytical techniques, traceability can only be achieved if an international standard of the same compound is available.

However, by using a relative primary method whose results are independent of the chemical structure, traceability can be established between two completely different compounds. A highly potent relative primary method is quantitative NMR (qNMR), which is increasingly used in the industry to determine the content of organic compounds.

Our site in Buchs has held a double accreditation (ISO/IEC 17025 and ISO Guide 34) for manufacturing CRMs by qNMR for almost seven years[1].

The organic TraceCERT reference materials are characterized by:

- Certified content by qNMR
- Superior level of accuracy, calculated uncertainties and lot-specific values
- Traceability to NISTRMS
- Comprehensive documentation delivered with the product (certification according to ISO Guide 31)

The primary advantage of ^{1}H quantitative NMR as a relative primary method is that the integrals of the proton signals are completely independent of the chemical structure.

Therefore, with a small set of NIST SRM traceable internal standards, we are able to certify basically any organic compound by measuring a gravimetrically produced solution of the analyte and the internal standard. The precisely determined mass-to-mass ratio then allows for the calculation of the analyte content with very high accuracy. Typical expanded uncertainties range from 0.5% down to 0.1%.

New Organic TraceCERT Products

Our offering of CRMs certified by qNMR comprises over 200 products and is continually expanded. See our most recent product additions in **Table 1**.

The complete portfolio can be found online at
sigma-aldrich.com/organiccrm

Cat. No.	Description	Package Size
75049	Capsaicin	50 mg
16826	Dicamba	100 mg
51436	2-Ethytoluene	100 mg
94495	Flutamide	50 mg
92585	Mono-Ethyl fumarate	50 mg
52451	Sodium benzoate	100 mg
04003	Tetradecan	100 mg
92359	DL-α-Tocopherol acetate	50 mg

Table 1. NEW Organic Neat CRMs TraceCERT for Chromatography

We also make our traceable internal standards for qNMR available to our customers working with quantitative NMR. The portfolio of internal standards suitable for ^{1}H qNMR is comprised of 15 products covering a wide range of ppm values and solubilities. Our website at **sigma-aldrich.com/qnmr** gives an overview of the standards available, including an example of a certificate and a graphic of all the NMR spectra. A PDF of the qNMR brochure is also downloadable on the website, explaining in detail how you can make your NMR instrument fit for quantitative, highly precise measurements.

Last year we also launched a first set of internal standards suitable for ^{31}phosphorous qNMR[2].

Find new product additions of both ^{1}H and ^{13}P qNMR standards in **Table 2**.

Cat. No.	Description	Intended Use	Package Size
92214	Potassium phosphate monobasic	31P q-NMR	1 g
74599	1,3,5-Trimethoxybenzene	1H q-NMR	1 g

Table 2. NEW Organic Neat CRMs TraceCERT for Quantitative NMR

References:

[1] Launch of a New Generation of Organic Certified Reference Materials. *Analytix, Vol 3,* **2010**.
[2] Traceable Organic Certified Reference Materials for ^{13}P Quantitative NMR. *Analytix, Vol 2,* **2015**.

The Importance of Analytical Instrumentation Qualification Using Certified Reference Materials

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Reliable Analytical Measurement

It is now universally accepted that reliable analytical measurement underpins the chemical industry; whether in pharmaceutical manufacture, food safety or paint composition, the need to ensure product quality and safety is paramount. Irrespective of the use of a Quality Standard, such as GMP or ISO 17025, failure to ensure valid analytical results can have massive impacts on an organization and its reputation for product quality.

Analysts spend a vast amount of time validating methods, running system suitability tests and performing QC and PT checks in order to demonstrate the validity of their method and results. All this work is based on the assumption that the instrument must be working correctly because all of the calibration, validation and QC checks have been done.

But what if the instrument itself was slightly faulty?

The starting point really should be that the analytical system itself is working properly because it has been calibrated using a Certified Reference Material (CRM).

An instrument that is working out of specification will frequently bias a result, in a reproducible way, and so may go unnoticed.

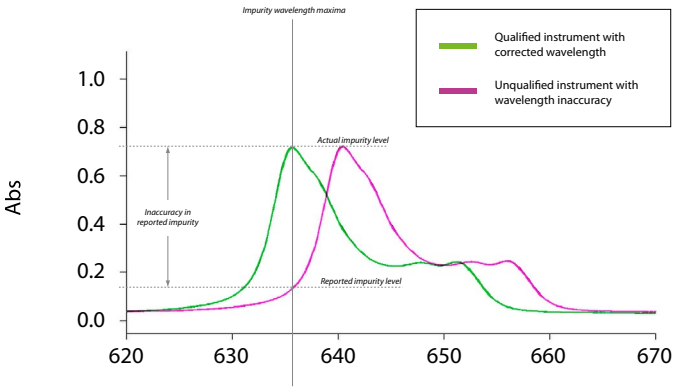

Figure 1. Wavelength Shift Causing an Incorrect Measurement

Figure 1 demonstrates the impact of an unqualified instrument on the reported impurity level in a product: a simple wavelength fault is causing an impurity to be reported at a level far lower than the true amount. This type of fault will allow the impurity to be quantified, but because of the fault, the sensitivity will be reduced and the impurity could incorrectly be below the detectable limit. The result is that a potentially lethal impurity is not reported.

Suitability for the Intended Application

More commonly referred to as being "fit for purpose", this concept stipulates that the instrument must be capable of performing the required task (purpose), and qualification is the method of proving that it is capable (i.e., fit for purpose).

The pharmaceutical industry requires a regime of regular instrument qualification and this, combined with change control, ensures that the equipment is "fit for purpose"[1]. Although ISO/IEC 17025:2005[2] only requires that the equipment is checked or calibrated before use, the fundamental difference is "fit for purpose". Defining the purpose is vital for instrument qualification; there are countless examples of attempts to develop methods which exceed the capability of the instrument. One such example is expecting repeatability of less than 1% when the 15-year-old instrument is only capable of less than 2%.

The flow diagram in **Figure 2** demonstrates that many of the questions raised during an investigation will cast doubt on the instrument suitability for the application and, without proper

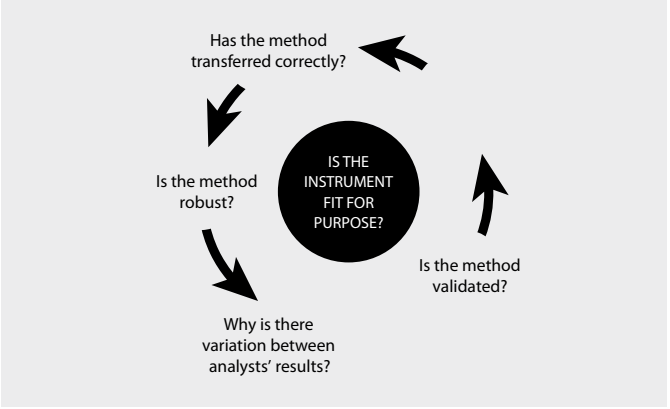

Figure 2. Key Questions for Out of Specification Investigations

qualification, it may be impossible to address these doubts. ISO/IEC 17025:2005 requires that all methods be suitable and validated without demanding Instrument Qualification. So when ISO 17025 is implemented in the context of cGMP regulations, all equipment MUST undergo a regular Performance Qualification (PQ). But outside the cGMP arena, no such PQ is mandated.

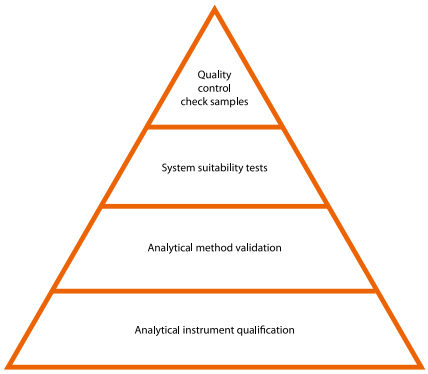

Figure 3. Data Quality Triangle

In the USP Monograph <1058> on Analytical Instrument Qualification[3], the concept of a Data Quality Triangle (DQT) is introduced (**Figure 3**). This outlines the necessity that Instrument Qualification forms the very foundation of all analytical measurement.

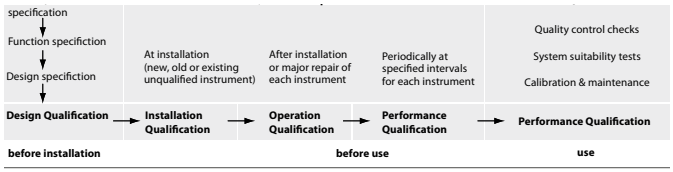

Figure 4. Timeline of Analytical Instrumentation Qualification (AIQ)

Once the purpose has been established, the Installation Qualification (IQ) phase should be conducted followed by the Operational Qualification (OQ) phase. IQ is where the non-variable requirements of the "purpose" are reviewed; for example, does the instrument have the desired sample capacity? The OQ phase demonstrates that the instrument will operate as expected; for example, no errors appear when the instrument is turned on.

Upon completion of the IQ/OQ phase, the Performance Qualification (PQ) phase begins. This is where the instrument is qualified to ensure that it meets the requirements of the analysis being carried out in the laboratory, i.e., running a CRM with certified wavelengths to prove that the instrument's wavelength is sufficiently accurate for the analysis that will be conducted.

Simple Instrument Performance Qualification

Unfortunately there are relatively few CRMs designed to qualify instruments. Many laboratory managers see no need and produce their own – after all they are simple solutions, or are they? No, they are not. During accreditation, auditors are increasingly looking to see that in-house CRMs are produced so that they generally achieve the same quality level as a commercially produced CRM. Following ISO/IEC 17034, which is the "standard" for the production of CRMs, is complex and labor intensive.

ISO/IEC 17025 is presently under revision, and it is likely that one of the changes will be to introduce a requirement for instrument qualification in addition to method validation. It seems that now is the time to start to implement regular instrument performance testing.

The benefit of using a CRM is that it provides instant traceability for the laboratory. Traceable instrument qualification adds confidence to all data generated.

To encourage routine instrument testing, the three basic rules are:

- Qualification should always be based on simplicity.
- If complex chemistry is used to validate a complex instrumentation, then any failure could be due to multiple factors and the true cause hidden.
- By simplifying the chemistry within the qualification process, it is easy to see which failures are due to the instrumentation and which are from the method.

The following Certified Reference Material kits are available to ensure simple and convenient instrument qualification.

Cat. No.	Description	Package Size
Kromega CRMs for HPLC		
Z803898	HPLC Autosampler Standards certified reference material	1 Kit
Z804339	HPLC Qualification Kit certified reference material	1 Kit
Z804002	UV Absorbance Linearity Standards certified reference material	1 Kit
Z804126	UV Low Wavelength Standards certified reference material	1 Kit
Z804223	UV Multi Wavelength Standards certified reference material	1 Kit
Kromega CRMs for UV/Vis Spectrophotometers		
Z804797	Enhanced UV/VIS Spectrophotometer Kit certified reference material	1 Kit
Z804452	UV Photometric Accuracy Standards certified reference material	1 Kit
Z804568	UV Resolution Standards certified reference material	1 Kit
Z804789	UV Spec Qualification Kit certified reference material	1 Kit
Z804665	UV Stray Light Standards certified reference material	1 Kit
Z804908	Wavelength, Stray Light and Resolution Kit certified reference material	1 Kit

More information can be found at **sigma-aldrich.com/jaytee**

These products have been developed out of years of experience in instrument service and qualification by Jaytee Biosciences, a highly respected reference material supplier and service provider to the UK's largest pharmaceutical and chemical companies. The current product range covers a variety of HPLC (or UHPLC) CRMs suitable for calibrating systems with any style of UV detection and a range of UV/Visible Spectroscopy CRMs, which meet the European Pharmacopeia's requirements for the calibration of UV/Vis spectrophotometers. Future additions to the range will include GC FID and other HPLC detectors.

References:

[1] Guidance for Industry, investigating Out-of-Specification (OOS) Test Results for Pharmaceutical Production. *U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research*, October **2006**.
[2] EN ISO/IEC 17025:**2005**.
[3] USP 38, NF 33 <1058> Analytical Instrument Qualification.

This article is based on a Quality Matters column published in Spectroscopy Europe Vol. 27 No. 6, p. 18 (2015); the original can be found at http://www.spectroscopyeurope.com/articles/quality-matters.

Total Security for Your pH Calibration

Certipur® Buffer Solutions & Substances in the Scope of ISO Guide 34 Accreditation

Bettina Straub-Jubb, Global Product Manager Titration
bettina.straub-jubb@merckgroup.com

Double Accreditation

Certipur buffer solutions and substances are now certified reference materials in the accreditation scope of the ISO Guide 34. This adds to our existing DIN EN ISO/IEC 17025 accreditation as a testing and calibration laboratory. Our double accreditation is the highest possible quality standard for certified reference buffers for pH calibration.

Buffers for Every Need – Now under the Accreditation Scope of the ISO Guide 34

- Ready-to-use solutions in practical, pre-portioned sachets – always a fresh solution
- Certified secondary pH reference materials as substances and solutions for extremely precise calibration and monitoring of instruments
- Buffer solutions measured at 20 °C or 25 °C to suit your needs
- Color-coded buffer solutions to avoid errors
- Buffers in Titripac® packaging to prevent contamination
- Ready-to-use certified buffer solutions acc. to EP & USP

Your Benefits

- Detailed certificate of analysis according to ISO Guide 31
- Excellent qualification with 5-point calibration for better accuracy
- Certified secondary buffers measured very accurately using differential potentiometry with two hydrogen platinum electrodes for instrument qualification
- Double traceability to NIST (National Institute of Standard & Technologies, USA) & PTB (Physical Technical Institute, Germany)
- Well prepared for audits with excellent documentation
- Secure and sophisticated packaging concepts


DAkkS
Deutsche Akkreditierungsstelle

Deutsche Akkreditierungsstelle GmbH

Entrusted according to Section 8 subsection 1 AkkStelleG in connection with Section 1 subsection 1 AkkStelleGBV

Accreditation

The Deutsche Akkreditierungsstelle GmbH attests that the reference material producer

Merck KGaA
Merck Millipore
Frankfurter Straße 250, 64293 Darmstadt

is competent according to the relevant clauses of ISO Guide 34:2009 and DIN EN ISO/IEC 17025:2005 to produce the following reference materials:

Conductivity standard solutions, element standard solutions, pH-reference solids and pH-reference solutions

The accreditation certificate shall only apply in connection with the notice of accreditation of 05.01.2016 with the accreditation number D-RM-15185-01 and is valid until 15.01.2018. It comprises the cover sheet, the reverse side of the cover sheet and the following annex with a total of 2 pages.

Registration number of the certificate: D-RM-15185-01-00

Berlin, 05.01.2016 — Andrea Valbuena, Head of Division — Translation issued 05.01.2016 — Head of Division

This document is a translation. The definitive version is the original German accreditation certificate.

What do ISO Guide 34 & DIN EN ISO/IEC 17025 mean? Trust.

Developed by the International Standardization Organization, ISO Guide 34 outlines requirements for qualification as a competent producer of reference materials. Our ISO Guide 34 accreditation is founded on several existing Merck KGaA Darmstadt, Germany, accreditations. One of these is DIN EN ISO/IEC 17025, which concerns the quality control stage. Together, these accreditations mean that you receive the most reliable products and services. In one word: trust.

With buffer solutions and substances from Merck KGaA, Darmstadt, Germany, you are always compliant with your Quality Assurance demands, and you will benefit from our stringent processes and experience for the analytical process in your Quality Control laboratory.

Use buffer solutions and solids from us, and you can always trust your pH results.

We take care of the complete process when producing a buffer, including sourcing or producing the raw material, the production of buffer solutions, and quality control, as well as transportation, correct storage, and after-sales support.

Discover more about our buffer portfolio at:
sigma-aldrich.com/certipur-ph-buffers

Fingerprint Application for Hypericum Perforatum

HPTLC: A Fast and Efficient Analysis Method for Medicinal Plants

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In the previous issue of Analytix (5/2016) (downloadable as pdf at **sigma-aldrich.com/analytix**), we showed the power of High-Performance Thin-Layer Chromatography (HPTLC) for the analysis of medicinal plants demonstrated by three applications for different compound classes present in *Ginkgo biloba*. It was demonstrated that the complex composition of a plant can be visualized, enabling one to quickly detect the ratio of the most important and regulated components or to reveal potential adulterations of the plant material.

In this issue, we will present as a further example an HPTLC application for St. John's Wort. *Hypericum perforatum L.*, known as Perforate St. John's-Wort, or simply St. John's Wort (SJW), is a flowering plant of the genus *Hypericum*. Extracts of SJW in herbal medicinal products have been traditionally used to treat a wide range of medical conditions, and

are commonly recommended as mild antidepressants[1–3]. *Hypericum* extracts contain a complex mixture of bioactive metabolites, such as naphtodianthrones (hypericin and pseudohypericin), phloroglucinols (hyperforin and adhyperforin), and flavonoids[4].

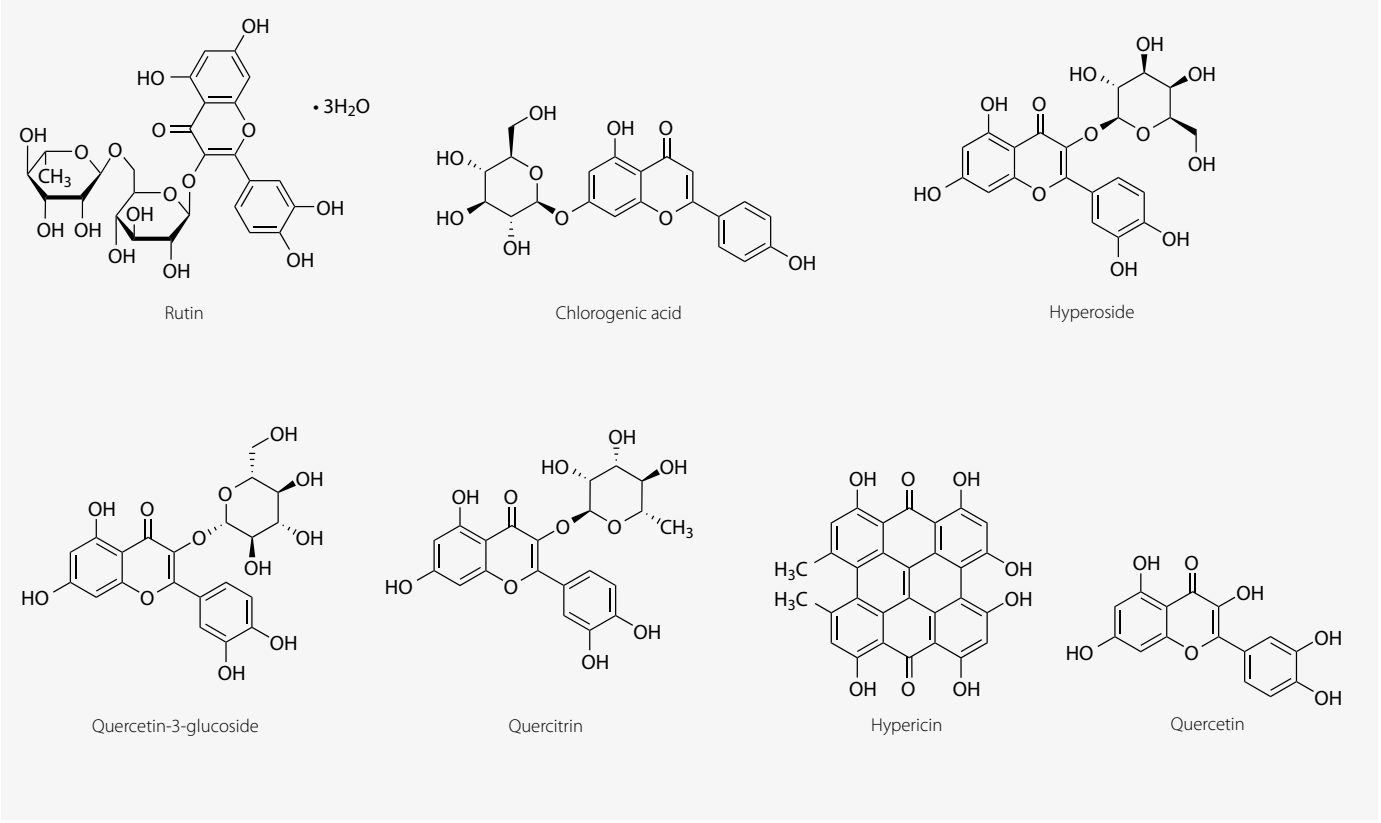

Figure 1. Chemical Structures of Components of *Hypericum Perforatum*

The zones seen in the HPTLC fingerprint of SJW (obtained by following the HPTLC method of the USP monograph on SJW[5]) were identified by comparison of the R_F values of the reference substances and the matching zones in the reference extract.

Recommended CAMAG Devices:
Automatic TLC Sampler 4 (ATS 4) or Linomat 5, Automatic Developing Chamber (ADC 2), TLC Visualizer, Chromatogram Immersion Device, TLC Plate Heater, and visionCATS

Derivatization Reagent:
Natural product reagent (NP) and polyethylene glycol solution (PEG)

Sample:
50 mg of extract are sonicated with 10 mL of methanol for 10 min. The solution is then centrifuged and the supernatant is used as test solution.

Standards:
Standard solutions were prepared in a concentration of 0.25 mg/mL in methanol.

Chromatography Following USP <203>[6]:
Stationary phase: HPTLC Si 60 F_{254} 20 x 10 cm.

Sample application:	2 µL each of test solution and standards are applied as 8 mm bands – 8 mm from lower edge of plate, 20 mm from the left edge using ATS 4.
Developing solvent:	Ethyl acetate dichloromethane acetic acid formic acid, water (100:25:10:10:11 *v/v/v/v/v*)
Development:	Development is performed with ADC 2, saturated for 20 minutes with the developing solvent (filter paper). Prior to the development, the plate is conditioned for 10 min to a relative humidity of 33% (with a saturated solution of $MgCl_2$).
Developing distance:	70 mm from lower edge of the plate
Plate drying:	5 min in a stream of cold air
Derivatization:	The plate is heated for 3 min at 100 °C with the TLC Plate Heater and immediately immersed (immersion time o s and immersion speed 3 cm/s) into NP reagent (1 g of NP in 200 mL ethyl acetate) then in PEG solution (10 g of polyethylene glycol 400 in 200 mL of dichloromethane) with the Chromatogram Immersion Device.
Evaluation:	Documentation under UV 366 nm after derivatization with the TLC Visualizer

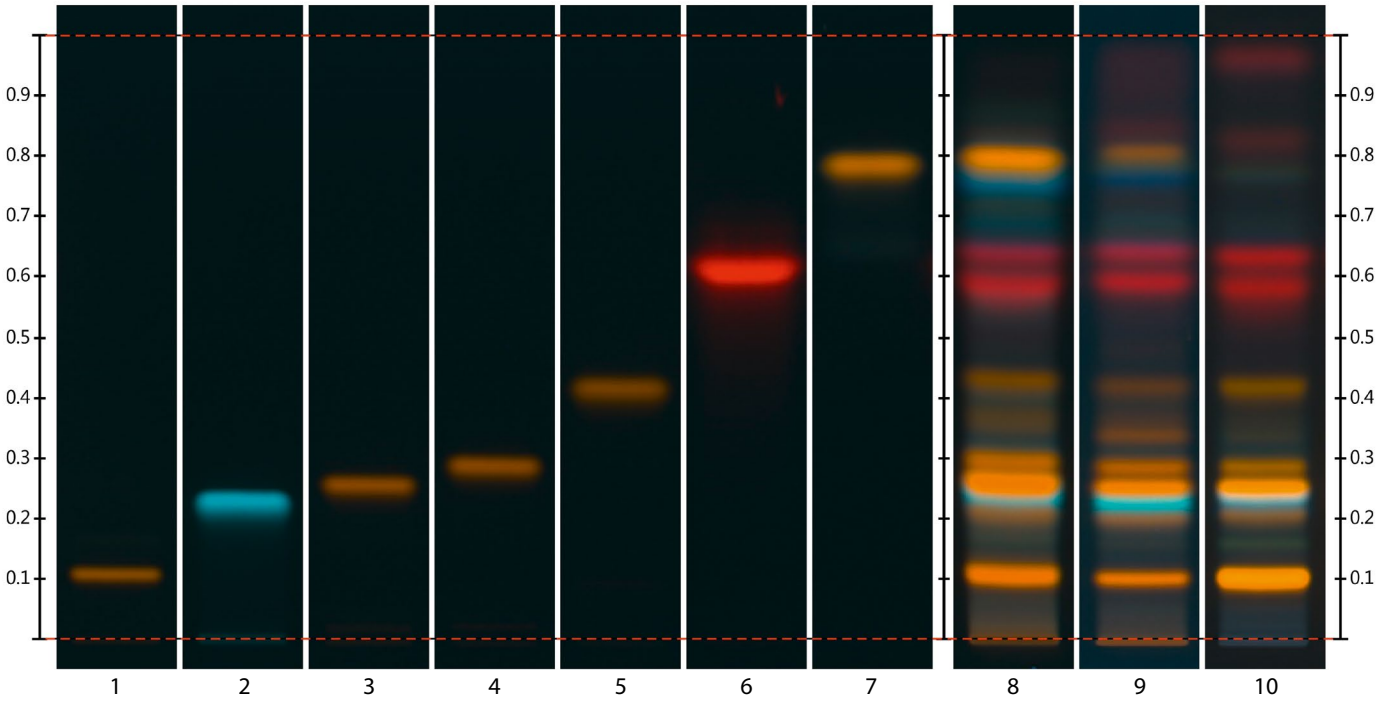

Figure 2. Chromatogram Under UV 366 nm After Derivatization.
Track 1: Rutin; Track 2: Chlorogenic Acid; Track 3: Hyperoside; Track 4: Quercetin-3-glucoside; Track 5: Quercitrin; Track 6: Hypericin; Track 7: Quercetin Dehydrate; Track 8: *Hypericum Perforatum* Extract Reference Material HWI Analytik, distributed by Sigma-Aldrich®; Track 9: SJW Dry Extract; Track 10: SJW Powdered Herb

Results:
Under UV 366 nm after derivatization (**Figure 2**) the fingerprint of SJW dry extract (track 9) and an SJW herb sample (track 10) look similar to that of the SJW reference extract from HWI Analytik, distributed by Sigma-Aldrich (track 8). Zones corresponding in color and position to the standards rutin (track 1), chlorogenic acid (track 2), hyperoside (track 3), quercetin-3-glucoside (track 4), quercitrin (track 5), hypericin (track 6) and quercetin dehydrate (track 7) can be identified in all samples and the extract reference material of *Hypericum perforatum* from Sigma.

Products Used:

Cat. No.	Description	Package Size
00500590	Chlorogenic acid	25 mg
00190585	Hypericin	10 mg
00180585	Hyperoside	25 mg
00200595	Quercetin	50 mg
16654	Quercetin 3-glucoside	10 mg
00740580	Quercitrin	25 mg
78095	Rutin	25 mg

Table 1. Reference Materials

Cat. No.	Description	Quantitative Markers Qualitative Markers	Package Size
05295001	Hypericum perforatum extract	Hypericin Pseudohypericin	150 mg

Table 2. Extract Reference Material Hypericum

Cat. No.	Description	Dimensions	Package Size
1.05642.0001	HPTLC glass plate Silica gel 60 F254	20 × 10 cm	50 Plates

Table 3. TLC Plates Used

Our reference materials and HPTLC plates used for this study are listed in **Tables 1–3**. A list of our complete offering of phytochemical standards can be found at **sigma-aldrich.com/medicinalplants** and all our extract reference materials, including an example certificate, can be viewed here: **sigma-aldrich.com/plantextracts**. All plant extract reference materials are delivered with a certificate giving the exact mass fractions for the quantitative markers. Additional qualitative markers are confirmed. A chromatographic method is also provided, including a chromatogram with peak assignation.

Our HPTLC plates enable significantly faster, high-precision results of outstanding quality. Learn more about the features of High-Performance Thin-Layer Chromatography plates at **www.merckmillipore.com/hptlc**

References:

[1] Linde, K.; Berner, M.; Egger, M.; Mulrow, C. *The British Journal of Psychiatry*, **2005**, 186, 99–107.

[2] Barnes, J.; Anderson, L.; Phillipson, J. *Journal of Pharmacy and Pharmacology*, **2001**, 53, 583–600.

[3] Muller, W.E.; Singer, A.; Wonnemann, M. *Schweiz Rundschau Med. Prax.*, **2000**, 89, 2111–2121.

[4] Kirakosyan, A.; Hayashi, H.; Inoue, K.; Charchoglyan, A.; Vardapetyan, H. Stimulation of the production of hypericins by mannan in Hypericum perforatum shoot cultures. *Phytochemistry*, **2000**, 53, 345–348.

[5] St. John's Wort: Monograph in USP 38-NF33. United States Pharmacopeial Convention, Rockville, MD, USA, **2015**.

[6] <203> High-performance Thin-layer Chromatography Procedure for Identification of Articles of Botanical Origin in USP 39-NF34. United States Pharmacopeial Convention, Rockville, MD, USA, **2016**.

New Analytical Standard for Abscisic Acid

And Other Additions to the Phytochemical Standards Range

Matthias Nold, Product Manager Reference Materials
matthias.nold@sial.com

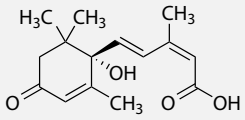

Figure 1. Abscisic Acid

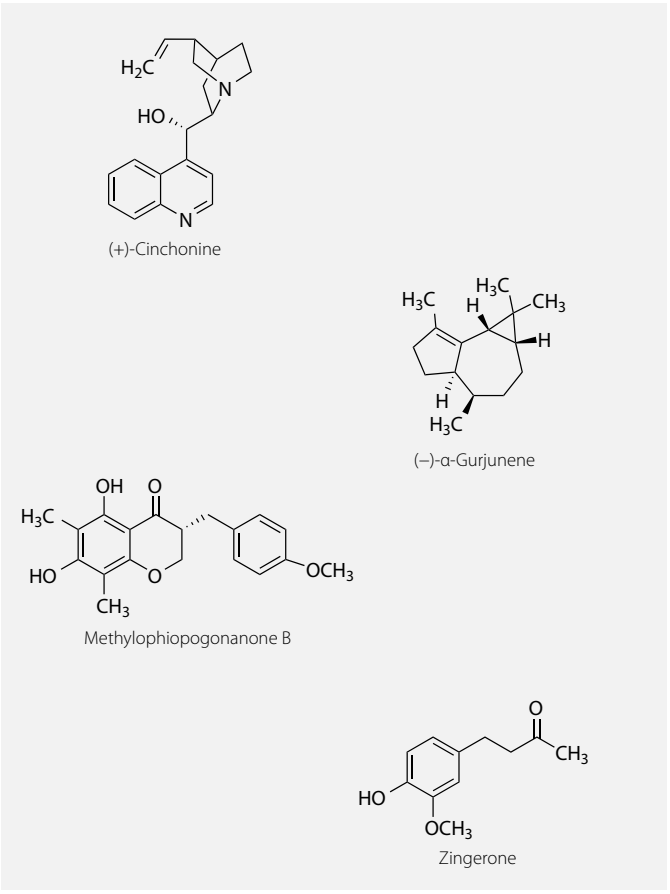

Figure 2. Other Recently Added Reference Materials of Phytochemicals

Abscisic acid is a plant hormone involved in the abscission of plant leaves and plant response to environmental stress. Many studies suggest beneficial effects on human health, including anti-inflammatory and anti-diabetic properties. Abscisic acid is therefore increasingly used in phytotherapy and as a dietary supplement.

We recently introduced a new reference material for abscisic acid. The content of the compound is tested by quantitative NMR (qNMR).

This product, along with the other new additions listed below, has increased the large portfolio of analytical standards and certified reference materials for plant constituents. It is currently comprised of more than 600 products.

On our website at **sigma-aldrich.com/medicinalplants**, you can find a complete listing of these products including a list of the most common medicinal plants and their active ingredients by plant genus, and you can either download or order a comprehensive brochure which includes product listings and selected chromatographic applications.

Cat. No.	Description	Package Size
90769	(+)-Abscisic acid	25 mg
19431	(+)-Cinchonine	50 mg
93449	(–)-α-Gurjunene	5 mg
92542	Methylophiopogonanone B	5 mg
88787	Zingerone	50 mg

Table 1. New Analytical Standards of Active Ingredients and Marker Substances for the Analysis of Herbal Medicinal Drugs

Aquastar™ – The Standard You Can Trust

Water Standards for Karl Fischer Titration

Bettina Straub-Jubb, Global Product Manager Titration
bettina.straub-jubb@merckgroup.com

Reliable Water Determination

New ISO 17025 Accreditation of our Karl Fischer QC Laboratory


DAkkS
Deutsche Akkreditierungsstelle

Deutsche Akkreditierungsstelle GmbH

Entrusted according to Section 8 subsection 1 AkkStelleG in connection with Section 1 subsection 1 AkkStelleGBV

Accreditation

The Deutsche Akkreditierungsstelle GmbH attests that the reference material producer

Merck KGaA
Merck Millipore
Frankfurter Straße 250, 64293 Darmstadt

is competent according to the relevant clauses of ISO Guide 34:2009 and DIN EN ISO/IEC 17025:2005 to produce the following reference materials:

Conductivity standard solutions, element standard solutions, pH-reference solids and pH-reference solutions

The accreditation certificate shall only apply in connection with the notice of accreditation of 05.01.2016 with the accreditation number D-RM-15185-01 and is valid until 15.01.2018. It comprises the cover sheet, the reverse side of the cover sheet and the following annex with a total of 2 pages.

Registration number of the certificate: D-RM-15185-01-00

Berlin, 05.01.2016 — Andrea Valbuena, Head of Division — Translation issued: 05.01.2016 — Head of Division

This document is a translation. The definitive version is the original German accreditation certificate.

Precise water determination is always a challenge. Using our Aquastar water standards together with the Aquastar reagents, you can always trust your results.

Within the European Union and worldwide, there has been increasing demand that analytical results become more transparent and comparable. To ensure this, reliable reference materials for analytical methods are necessary. The Aquastar line offers an excellent series of standards for the Karl Fischer Titration method, qualified in our DIN EN ISO/IEC 17025 accredited calibration and testing laboratory at Merck KGaA, Darmstadt, Germany.

In addition to their use in monitoring Karl Fischer equipment and performing titer determinations, they are also used to test measurement results in order to evaluate their accuracy. Our Aquastar standards are manufactured under the strictest controls to fulfill the existing requirements of a reference material.

By using Aquastar Water Standards together with our Aquastar reagents, you are prepared for audits and inspections from internal and external authorities.

The significance of the Karl Fischer Titration is emphasized by the fact that it has been included in the most important Pharmacopeias, American Standard Methods (ASTM), DIN EN ISO Guidelines, Association of Official Agricultural Chemist Methods (AOAC) and other requirements.

Water determination according to Karl Fischer Titration is one of the most precise and rapid methods to determine water content in a range from 10 ppm to 100%. It can be used for a wide variety of samples.

Benefits of Aquastar Karl Fischer Standards

Reliability: High accuracy

Transparency: Adheres to the requirements of the DIN EN ISO/IEC 17025 accreditation

Documentation: Certificate according to the ISO Guide 31 guideline for reference materials

Precision: High batch-to-batch consistency

Easy to use: Convenient packaging

Cat. No.	Description	Packaging
1.12939.0010	Aquastar Lactose Standard 5%, for volumetry and KF oven method	10 g PE bottle
1.06664.0100	Aquastar Sodium tartrate dihydrate 15.66%, volumetric standard for water determination acc. to Karl Fischer	100 g PE bottle
1.88050.0010	Aquastar Water Standard 0.01%, 1 g contains 0.1 mg H_2O	10 x 8 mL glass ampoule
1.88051.0010	Aquastar Water Standard 0.1%, 1 g contains 1 mg H_2O	10 x 8 mL glass ampoule
1.88052.0010	Aquastar Water Standard 1%, 1 g contains 10 mg H_2O	10 x 8 mL glass ampoule
1.88054.0005	Aquastar Water Standard Oven 1%, solid standard for KF oven method	5 g glass bottle
1.88055.0010	Aquastar Water Standard Oil, standard for oil samples for coulometric Karl Fischer titration (15–30 ppm)	10 x 8 mL glass ampoule

Table 1. Aquastar Water Standards Ordering Information

Different Grades to Meet Your Needs!

High-Purity Reagents for Trace Analysis

Patric Klein, Global Product Manager Trace Elemental Analysis
patric.klein@merckgroup.com
Daniel Weibel, Global Product Manager Trace Organic Analysis & Sensorics
daniel.weibel@sial.com

Sample preparation in instrumental trace analysis requires reagents with low impurity levels. The reagents can have an important impact on the outcome of the measurement, since in modern instrumental analysis, any impurity could disturb the measurement.

We offer a wide range of reagents for the different applications.

Do you perform inorganic trace elemental analysis? We offer high-purity acids for wet digestion – Suprapur® and Ultrapur®.

Do you perform HPLC or LC-MS analysis? We offer high-purity reagents – LiChropur®.

Suprapur	Ultrapur	LiChropur
High-purity acids and bases	High-purity acids	Acids, bases & salts
Elemental trace analysis (e.g., AAS, ICP-OES)	Elemental ultra-trace analysis (e.g., ICP-OES, ICP-MS)	HPLC and LC-MS reagents
ppm – ppb	ppb – ppt	ppm – ppb

Suprapur Acids and Bases

Suprapur acids and bases are suitable for trace analysis in the ng/g (ppb) range.

Suprapur acids are packaged in borosilicate or extra-pure PE bottles. The material minimizes any elemental impurity of the acid, so the specification of the unopened bottle is kept during the minimum shelf life. The bottles are cleaned and pre-conditioned before filling. Quality control is done after filling. This gives you the insurance that the certified batch values are those values of the filled acid. Suprapur reagents are packed in a stable outer box. Hydrogen peroxide Suprapur is packed in a black bottle to protect it against light. The bottle is made of extra-pure PE material to avoid any contamination. To make it safer for you, hydrogen peroxide bottles are closed with the SafetyCap. The SafetyCap with the PTFE membrane releases the pressure, but avoids any contamination.

Cat. No.	Description	Content	Packaging
1.00066.0250	Acetic acid 100% Suprapur	250 mL	Glass bottle
1.00066.1000		1 L	Glass bottle
1.05428.0250	Ammonia solution 25% Suprapur	250 mL	PE bottle
1.05428.0500		500 mL	PE bottle
1.05428.1000		1 L	PE bottle
1.05428.2500		2.5 L	PE bottle
1.00765.0050	Boric acid Suprapur	50 g	PE bottle
1.00765.0500		500 g	PE bottle
1.11670.0250	Formic acid 98–100% Suprapur	250 mL	Glass bottle
1.11670.1000		1 L	Glass bottle
1.00306.0250	Hydrobromic acid 47% Suprapur	250 mL	Glass bottle
1.00306.1000		1 L	Glass bottle
1.00318.0250	Hydrochloric acid 30% Suprapur	250 mL	PE bottle
1.00318.0500		500 mL	PE bottle
1.00318.1000		1 L	PE bottle
1.00318.2500		2.5 L	PE bottle
1.00335.0250	Hydrofluoric acid 40% Suprapur	250 mL	PE bottle
1.00335.0500		500 mL	PE bottle
1.00335.1000		1 L	PE bottle
1.00335.2500		2.5 L	PE bottle
1.07298.0250	Hydrogen peroxide 30% Suprapur	250 mL	PE bottle
1.07298.0500		500 mL	PE bottle
1.07298.1000		1 L	PE bottle
1.00441.0250	Nitric acid 65% Suprapur	250 mL	Glass bottle
1.00441.1000		1 L	Glass bottle
1.00489.0100	Oxalic acid dihydrate Suprapur	100 g	PE bottle
1.00517.0250	Perchloric acid 70% Suprapur	250 mL	Glass bottle
1.00517.1000		1 L	Glass bottle
1.00552.0250	Ortho-Phosphoric acid 85% Suprapur	250 mL	PE bottle
1.00552.0500		500 mL	PE bottle
1.00552.1000		1 L	PE bottle
1.00552.2500		2.5 L	PE bottle
1.05589.0250	Sodium hydroxide solution 30% Suprapur	250 mL	PE bottle
1.05589.0500		500 mL	PE bottle
1.05589.1000		1 L	PE bottle
1.05589.2500		2.5 L	PE bottle
1.00714.0250	Sulfuric acid 96% Suprapur	250 mL	Glass bottle
1.00714.1000		1 L	Glass bottle

Table 1. Ordering Information Suprapur

Ultrapur Acids

Ultrapur reagents are preferred for ultra-trace analysis in the pg/g (ppt) range.

Ultrapur reagents are produced by sub-boiling distillation. The slowly distilled reagents subsequently have the lowest possible trace impurities. Ultrapur reagents are exclusively packaged in pre-conditioned PFA (fluorpolymer) bottles. This material meets the highest demands of all users for ultra-trace instrumental analysis, e.g. ICP-MS. Ultrapur reagents are packed in a stable outer box.

Cat. No.	Description	Content	Packaging
1.01514.0250	Hydrochloric acid 30% Ultrapur	250 mL	PFA bottle
1.01514.0500		500 mL	PFA bottle
1.01514.1000		1 L	PFA bottle
1.01513.1000	Hydrofluoric acid 48% Ultrapur	1 L	PFA bottle
1.06097.1000	Hydrogen peroxide 31% Ultrapur	1 L	PFA bottle
1.01518.0250	Nitric acid 60% Ultrapur	250 mL	PFA bottle
1.00318.0500		500 mL	PFA bottle
1.00318.1000		1 L	PFA bottle
1.01516.0250	Sulfuric acid 96% Ultrapur	250 mL	PFA bottle
1.01262.0500	Water Ultrapur	500 mL	PE bottle
1.01262.1000		1 L	PE bottle

Table 2. Ordering Information Ultrapur

LiChropur Reagents

Trust the quality of our LC-MS reagents. Choose LiChropur.

New LC-MS systems have raised the bar on the purity expectations of chemicals used for sample preparation, mobile phases and post-column additives. Alkali ions, plasticizers and surfactants interfere strongly with LC-MS by causing higher background noise and the formation of adducts.

Sodium and potassium ions are especially likely to form adducts with analyte molecules. This leads to complex mass spectra, which causes time-consuming data evaluation. The content of trace metals is in the low ppb range for the new LC-MS grade acids and bases. Because of that, the risk of adduct formation in the ion source is minimized. The packaging of the acids and bases in borosilicate bottles also prevents the leaching of alkali ions out of the glass. The content of the potentially complex-forming ions – aluminum, copper and iron – is also specified at a low ppb level.

Cat. No.	Name	Description	Package Size
5.33001.0050	Acetic acid	100% for LC-MS LiChropur	50 mL
5.33002.0050	Formic acid	98–100% for LC-MS Lichropur	50 mL
5.33003.0050	Ammonia solution	25% for LC-MS LiChropur	50 mL
5.33004.0050	Ammonium acetate	For LC-MS LiChropur	50 mL
5.33005.0050	Ammonium hydrogen carbonate	For LC-MS LiChropur	50 mL

Table 3. Ordering Information for LiChropur

Reserpine is used as the reference substance to quantify possible impurities in LiChropur reagents. For electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) in the positive mode, the specified amount of reserpine is 2 ppb for acids and bases and 20 ppb for salts. In the negative mode, the specified amount of reserpine is 20 ppb. The reserpine test is performed by diluting 2.5% (v/v) acid or base or else 2.5% (w/v) salt in 50/50 (v/v) acetonitrile/water. Every lot produced is analyzed via flow injection analysis mass spectrometry (FIA-MS). The dissolved reagent and the appropriate reserpine reference solutions are brought to the ion source of the MS, free of contamination by syringe pumps. The total ion chromatogram (TIC) is summed over three minutes. The relative intensities of the detected masses are compared with the reserpine signal.

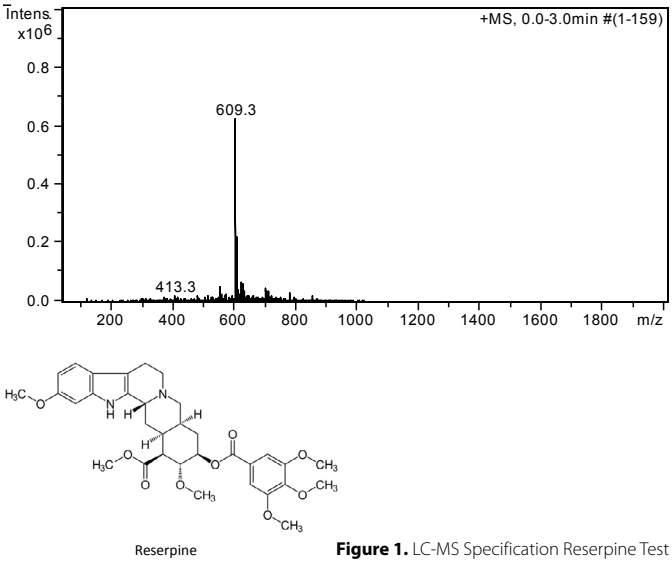

Figure 1. LC-MS Specification Reserpine Test

Only highly pure reagents allow high signal-to-noise ratios. LiChropur reagents are specifically designed to meet the requirements of high purity and consistency.

Learn more at **sigma-aldrich.com/lcms-reagents**

Atto Dyes™

For Excellent Fluorescence Application

Dr. Monika Bäumle, Global Product Manager, Instrumental Analysis Franchise
monika.baeumle@sial.com

Fluorescent techniques are widespread and fast-growing analytical methods used in life science. They allow sensitive and selective investigation of biological processes, diagnostic screening, kinetics, and conformational studies. Research is evolving from identification of a large number of target molecules to isolation and investigation at the single molecule level. Activated fluorescent dyes are routinely used to tag proteins, nucleic acids, and other biomolecules for use in life science applications including fluorescence microscopy, flow cytometry, fluorescence *in situ* hybridization (FISH), receptor binding assays, and enzyme assays. The Atto dyes are a series of fluorescent dyes that meet all the critical needs of modern fluorescent technologies, including:

- Stability – Atto 655 and Atto 647N, for example, are photostable and highly resistant to ozone degradation
- Long Signal Lifetimes – Signal decay times of 0.6–4.1 nanoseconds allow timegate studies to reduce autofluorescence background and scattering
- Reduced Background – Several Atto dyes employ excitation wavelengths greater than 600 nm, reducing background fluorescence from samples, and Rayleigh and Raman scattering
- Selection – Atto dyes have strong fluorescent signals that cover visible and near-IR emission wavelengths

Alternatives to Common Fluorophores

With the extensive selection available, Atto dyes can replace more commonly used fluorescent dyes. There are Atto dyes suitable for use with any common excitation light source.

Fluorophore	Recommended Atto Dye Alternative
Alexa Fluor® 488	Atto 488
FITC	
FAM™	
JOE™	Atto 520
TET™	
Alexa Fluor 532	Atto 532
HEX™	Atto 532, Atto Rho6G
TAMRA™	Atto 550
Cy®3	Atto 550
Cy3.5	Atto 565
ROX™	Atto 565, Atto Rho11
Alexa Fluor 594	Atto 590 , Atto 594
Texas Red®	Atto 590
Alexa Fluor 633	Atto 633, Atto Rho14
Cy5	Atto 647, Atto 647N , Atto 655
Alexa Fluor 647	Atto 647, Atto 647N, Atto 655
Cy5.5	Atto 680, Atto 700

Table 1. Recommended Atto Dye Alternatives to Commonly Used Dyes

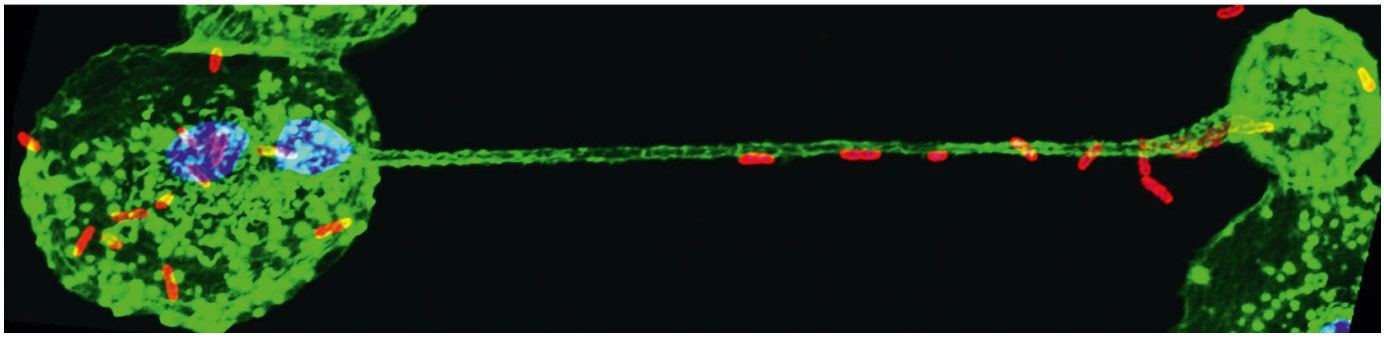

Figure 1. Confocal Laser Scanning Micrograph (CLSM) of human peripheral blood monocytes differentiated to macrophages, and infected with a red fluorescent Serratia liquefaciens. A macrophage tries to capture bacteria by means of a long pseudopod. Bacteria seem to walk along this structure. Fixed cells were permeabilized with Triton X-100. Atto-488 phalloidin (Sigma-Aldrich®) binds polymerized F-actin, used to identify actin filaments and fibers. Preparations were mounted in Fluoroshield™ mounting medium containing DAPI (Sigma-Aldrich). Series of optical sections were obtained with a NIKON A1R confocal scanning laser microscope equipped with a Nikon A1 digital camera, and 403nm, 488nm, and 561nm lasers. Image was kindly provided by Prof. Jose Ramos Vivas.

Atto Dyes – Superior Tools for Super-Resolution Microscopy Application

Recent developments in microscopy application, such as STED microscopy, enable resolutions down to 10 nm. These applications require fluorescent dyes that fulfill superior photo-physical criteria. Some Atto dyes, like Atto 488, Atto 647N, and Atto 655, have proven to be suitable for techniques such as PALM, dSTORM, STED, etc.[1–6]

Cat. No.				λ_{abs}	ε max	λ_{em}	η_{em}	τ_{em}
Free Acid	NHS Ester	Maleimide	Atto Dye	[nm]	[M^{-1} cm^{-1}]	[nm]	[%]	[ns]
89313	89204	89740	Atto 390	390	24,000	479	90	3.8
56759	16805	49349	Atto 425	436	45,000	484	90	3.5
50712	53404	55607	Atto 465	453	75,000	508	55	2.2
41051	41698	28562	Atto 488	501	90,000	523	80	3.2
16951	00379	41022	Atto 495	495	80,000	527	45	2.4
70706	77810	16590	Atto 520	516	110,000	538	90	3.8
06699	88793	68499	Atto 532	532	115,000	553	90	3.8
42424	92835	30730	Atto 550	554	120,000	576	80	3.2
75784	72464	18507	Atto 565	563	120,000	592	90	3.4
70425	79636	39887	Atto 590	594	120,000	624	80	3.7
08637	08741	08717	Atto 594	601	120,000	627	85	3.5
78493	93259	41061	Atto 610	615	150,000	634	70	3.3
40049	18708	94464	Atto 611X	611	100,000	681	35	2.5
92716	67351	49728	Atto 620	619	120,000	643	50	2.9
18620	01464	n/a	Atto 633	629	130,000	657	64	3.2
97875	07376	41784	Atto 647	645	120,000	669	20	2.3
04507	18373	05316	Atto 647N	644	150,000	669	65	3.4
93711	76245	80661	Atto 655	663	125,000	684	30	1.9
16851	04022	01407	Atto 665	663	160,000	684	60	
94875	75999	04971	Atto 680	680	125,000	700	30	1.8
30674	16986	50611	Atto 700	680	120,000	719	25	1.5
47156	93725	n/a	Atto 725	729	120,000	752	10	0.5
91394	59808	n/a	Atto 740	740	120,000	764	10	0.6

Table 1. Ordering Information for Selected Atto Dye Products
λ_{abs} – longest-wavelength absorption maximum
ε_{max} – molar extinction coefficient at the longest-wavelength absorption maximum
λ_{em} – fluorescence maximum
η_{em} – fluorescence quantum yield
τ_{em} – fluorescence decay time

For further information, please visit **sigma-aldrich.com/atto**

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[1] Yang, X.; Tzeng, Y-K.; Zhu, Z.; Huang, Z.; Chen, X.; Liu, Y. et al. Sub-diffraction imaging of nitrogen-vacancy centers in diamond by stimulated emission depletion and structured illumination. *RSC Adv. 4 (22), DOI: 10.1039/c3ra47240j*, **2014**, 11305.

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[3] Horrocks, M.H.; Palayret, M.; Klenerman, D.; Lee, S.F. The changing point-spread function. Single-molecule-based superresolution imaging. *Histochem Cell Biol 141 (6), DOI: 10.1007/ s00418- 014-1186-1*, **2014**, 577–585.

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Abberior® Dyes

For Superior Super-Resolution Microscopy Application

Dr. Monika Bäumle, Global Product Manager, Instrumental Analysis Franchise
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Optical microscopy is a powerful and multipurpose analytical tool. However, the conventional microscopy techniques suffer from low spatial resolution, mainly due to the diffraction of light. In recent years, revolutionary advances have enabled researchers to overcome the resolution barrier given by the diffraction limit. The Nobel Laureate Prof. Stefan Hell has invented the revolutionary STED Microscope[1] that for the first time enables a microscopic resolution limit below the theoretical limit of resolution and permits more detailed studies in cellular processes.

Conventional light microscopy enables a resolution limit of about 250 nm in the x- and y-direction and 450–700 nm in the z-direction. Super-resolution techniques have overcome the resolution limit (point-spread function) by at least a factor of 2. Additional new microscopy concepts have been developed in recent years. These super-resolution microscopy principles are based on several technological approaches.

Established techniques are:

- STED (Stimulated emission depletion)
- GSDIM (Ground state depletion)
- PALM (Photoactivated localization microscopy)
- STORM (Stochastic optical reconstruction microscopy)
- RESOLFT [Reversible saturable optical (flurorescence) transitions], (RSFPs)

The choice of fluorescent probe is critical in super-resolution microscopy. The risk of photobleaching, the state of the fluorescent probe, and the probe's brightness are some of the factors which are important in choosing a probe for super-resolution. In addition, experiments using multiple labels require more consideration.

Depending on the super-resolution method, further photophysical criteria of the probe must be fulfilled. MilliporeSigma offers Abberior dyes and derivatives that are exceptionally well suited for confocal microscopy, epifluorescence imaging and single molecule applications. All fluorescence applications which depend on a good signal-to-noise ratio and low background benefit from the novel Abberior dyes. Manufactured by Abberior GmbH, STAR, CAGE and FLIP dyes as well as RSFPs are exceptionally bright and photostable. They provide excellent performance in STED microscopy (STAR dyes) and are also optimized in photoswitching for RESOLFT (RSFPs) and PALM/STORM imaging (CAGE and FLIP dyes). They are the only commercially available dyes that are tailored specifically to the needs of super-resolution microscopy.

Benefits

- Optimized for brightness and very low background
- Optimized switching behavior being the key for super-resolution
- All markers are tested for different super-resolution methods
- Abberior STAR for STED, confocal and epifluorescence imaging

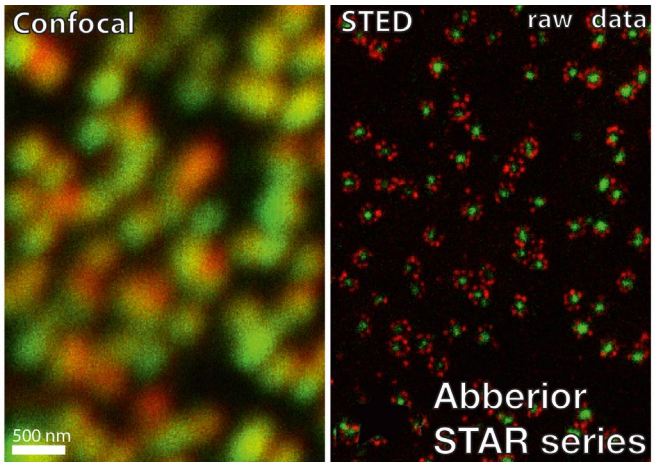

Figure 1. Super-resolution microscopy allows new insights into the structure of the nuclear-pore complexes on the nuclear membrane (confocal microscopy (left) and STED (right))

- Abberior CAGE & FLIP for PALM, STORM and GSDIM
- Abberior dyes are recommended by renowned microscope vendors
- Proprietary, IP-protected products
- Detailed characteristics of the dyes provided, e.g., optimal STED wavelength

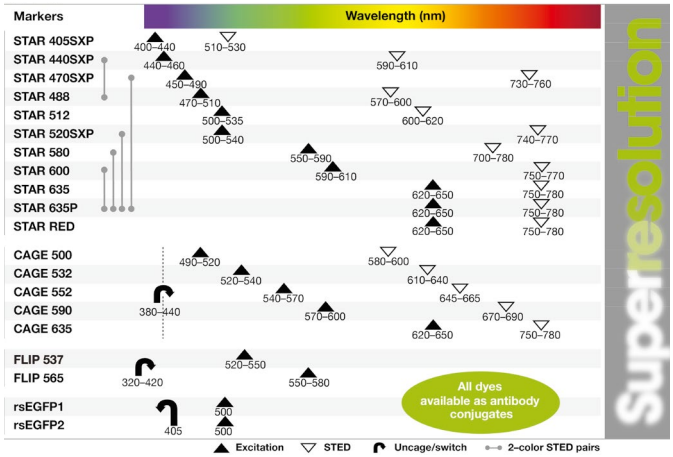

Figure 2. Wavelength Overview

Reference:

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Cat. No. NHS Activated	Cat. No. Maleimide Activated	Dyes	Description	Absorption Maximum/λmax	Extinction Coefficient, ε(λ)	Fluorescence Maximum, λfl	Recommended STED
44254	92546	Abberior CAGE 500	for single-molecule switching microscopy (e.g. PALM, STORM, GSDIM)	300 nm (caged, pH 7); 501 nm (uncaged, pH 7)	85,000–88,000 $M^{-1}cm^{-1}$ (pH 7, uncaged)	524 nm (pH 7), 523 nm (MeOH)	595–615 nm
38977	95705	Abberior CAGE 532	for single-molecule switching microscopy (e.g. PALM, STORM, GSDIM)	304 nm (caged, PBS, pH 7); 518 nm (uncaged, pH 7)	29,000 $M^{-1}cm^{-1}$ (pH 7, uncaged)	541 nm (pH 7)	610–640 nm
94822	92545	Abberior CAGE 552	for single-molecule switching microscopy (e.g. PALM, STORM, GSDIM)	300 nm (caged, pH 7); 552 nm (uncaged, pH 7)	66,000 $M^{-1}cm^{-1}$ (pH 7, uncaged)	574 nm (pH 7)	650–670 nm
77958	no	Abberior CAGE 590	for single-molecule switching microscopy (e.g. PALM, STORM, GSDIM)	caged: 295 nm (pH 7); uncaged 586 nm (pH 7)	51,000 M-1cm-1(MeOH)	607 nm (pH 7)	685–715 nm
79189	92544	Abberior FLIP 565	for single-molecule switching microscopy (e.g. PALM, STORM, GSDIM)	314 nm (PBS, pH 7.4)	30,800 $M^{-1}cm^{-1}$(MeOH), 22,700 M-1cm-1 (PBS, pH 7.4)	580 nm (PBS, pH 7.4)	590–620 nm
68221	38361	Abberior STAR 440SX	for long Stokes STED and 2-color STED application	430 nm (MeOH), 437 nm (PBS, pH 7.4)	30,400 $M^{-1}cm^{-1}$(MeOH), 22,700 $M^{-1}cm^{-1}$ (PBS, pH 7.4)	501 nm (MeOH), 515 nm (PBS, pH 7.4)	590 nm
95348	no	Abberior STAR 470SX	for long Stokes STED and 2-color STED application	475 nm (MeOH), 477 nm (PBS, pH 7.4)	86,000 $M^{-1}cm^{-1}$(MeOH)	609 nm (MeOH), 627 nm (PBS, pH 7.4)	740–770 nm
61048	no	Abberior STAR 488	for STED application	501 nm (PBS, pH 7.4)	74,000 $M^{-1}cm^{-1}$(MeOH)	524 nm (PBS, pH 7.4)	585–605 nm
38922	3004	Abberior STAR 512	for STED application	517 nm (MeOH), 512 nm (PBS, pH 7.4)	74,000 $M^{-1}cm^{-1}$(MeOH)	536 nm (MeOH), 530 nm (PBS, pH 7.4)	590–620 nm
38377	no	Abberior STAR 580	for STED application	587 nm (MeOH), 583 nm (PBS, pH 7.4)	64,300 $M^{-1}cm^{-1}$(MeOH)	609 nm (MeOH), 605 nm (PBS, pH 7.4)	690–720 nm
30558	96013	Abberior STAR 635	for STED application	639 nm (MeOH), 634 nm (PBS, pH 7.4)	63,000 $M^{-1}cm^{-1}$(MeOH)	659 nm (MeOH), 654 nm (PBS, pH 7.4)	740–770 nm
7679	no*	Abberior STAR 635P	for STED application	635 nm (MeOH), 634 nm (PBS, pH 7.4)	80,000 $M^{-1}cm^{-1}$(water)	655 nm (MeOH), 654 nm (PBS, pH 7.4)	740–770 nm

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